LABORATORY BIOSAFETY GUIDEBOOK

FOR BSL1 AND BSL2 CLINICAL AND RESEARCH LABORATORIES IN PAKISTAN

BY

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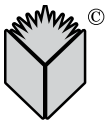

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Contents

Preface

Advancement in life sciences and research has increased the use of hazardous biological materials in laboratories all over the country. The increasing use of these infectious organisms demands a proper and safe methodology for their manipulation.

This Manual has been compiled to serve as a guideline so as to provide information regarding work practices, policies, protocols and systems to work safely at BSL-1 and BSL-2 Clinical Laboratories and at Research facilities in Pakistan. According to the prevailing conditions of advancement in health care centers and enormous increase in the number of clinical laboratories all over the country, there is an immediate need to institute a regular level of Biosafety and Biosecurity environment. This manual shall help spread the knowledge from grass root level to build the Biosafety policies for laboratories in order to keep our local and regional laboratories working condition suitable according to International Biorisk Management standards (CWA 15793).

At present, the major hurdle in maintaining Biosafety in Pakistan is the lack of institutional level risk management and Biosafety professionals in this part of the planet. In addition, the training facilities are not up to work. So to overcome these hurdles, institutions shall determine specific and suitable biocontainment level for their laboratories, and then manage Biosafe and Biosecure practices accordingly, and impart training with reference to Biosafety and Biosecurity. Biosafety guidelines must be followed with true spirit, making every possible method which should be cost effective and able to be implemented in these laboratories according to the risk assessment performed in line with the work performed in present and future particularly.

It will be worth mentioning here that this manual is written in compliance with the local/regional working capacity and requirements.

Furthermore, it is felt that there was a gap with respect to the availability of Biosafety manual in clinical and research laboratories at BSL-1 and BSL-2. for quick reference and guidance.

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- Dr. Anwar Nasim, Patron in Chief, PBSA
- Dr. Aamir Ikram, Founder PBSA
- Dr. Erum Khan, President PBSA

Other important members who assisted in compiling different parts of this manual are:

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- Ms. Shahida Qureshi
- Robert Heckert
- Jennifer Gaudisio
- Sumi Pranajapki
- Jonathan Richmond

INTRODUCTION

I. BIOSAFETY

Biosafety is the application of knowledge, techniques and equipment in order to prevent personal, laboratory and environmental exposure to potentially infectious agents or Biohazardous materials. Biosafety defines the control conditions under which infectious agents can be safely manipulated. The goal of Biosafety is to lock up biohazards and to reduce the possible exposure of the laboratory worker, persons outside of the laboratory, and the environment, to potentially infectious agents that are handled or stored in laboratory; it also encompasses the safety of lab personnel from any hazardous chemicals or lab equipments.

Biosafety is related to several fields as: ecology, chemistry, exobiology, medicine, agriculture, and human health.

II. EMERGENCE OF BIOSAFETY IN PAKISTAN

In response to International obligations imposed on global community, being a member of UN, and in compliance to IHR, work on these issues was started, keeping in view, the National concern with reference to Biosafety, which were mainly due to enormous increase in the number of Diagnostic Laboratories, Blood Banks and Medical facilities like hospitals in all mega cities of Pakistan.

The work with infectious agents in public and private research centers, animal care and agricultural facilities has expanded enormously.

A. Biorisk Management

The awareness with regard to Biosafety and Biosecurity issues was lacking, there was very little information about different aspects of Biorisk management, which comprises:

1. Risk assessment

2. Handling of potentially infectious agents
3. Disposal of laboratory wastes
4. Universal precautions

And as such biological risk spectrum was at large in respect of naturally occurring diseases, accidents (Laboratory Acquired Infections), no reporting protocol for LAIs, lack of awareness and negligence, lack of training, also to take into account the deliberate misuse of biological agent or toxic materials.

All these issues, obligations and concerns resulted in the evolution of Pakistan Biological Safety Association (PBSA).

Formed recognition of PBSA was awarded at COMSTECH Islamabad in a high level meeting of experts with national core group of life sciences (NCCLS) to launch PBSA. In continuation of this, PBSA was announced on March 29, 2008 under the dynamic leadership of Dr. Anwar Nasim with the support of NCCLS.

The vision of this association is to help ensure Biosafety through the promotion of best practices, standards and code of conduct in life sciences that deal with pathogenic microorganisms, biological toxins, and rDNA.

This Biosafety manual is a part of the aims and objectives of PBSA to introduce Biosafety and Biosecurity in Pakistan. It will also serve the increasing demand of laboratory professionals to take help regarding Biosafety and Biosecurity Issues on the basis of local and regional needs and requirements.

III. THE LABORATORY-ACQUIRED INFECTIONS

Greatest need of caution exists in laboratory because it is a place that deals with infectious materials, contaminated samples from the patients and other biomedical hazardous equipments. Thus, if laboratory practices and procedures are not carefully and safely handled then it can serve as a source point for the transmission of various infections and diseases to other lab workers, persons in hospital premises, community and to the environment. Lab-Acquired Infections (LAIs) continue to occur both in developed and under developed countries, but unfortunately most of them go unreported because of the failure of lab workers to do so, and due to lack of any infrastructure, as many countries do not have legal obligations to implement it.

LAIs can be the result of any of the following mishandled practices:

- The transmission of infectious agent to laboratory worker.
- The transmission of infectious agent from laboratory worker to other individuals.
- By the direct transmission of agents released from laboratory.

A. Most Common Laboratory-Acquired Infections

Laboratory-Acquired Infections (LAIs) occur due to a wide variety of bacteria, viruses, fungi, and parasites. The most common are narrated below:

1. ***Bacterial*** *Brucella* species, *Shigella* species, *Salmonella* species, *Mycobacterium tuberculosis*, and *Neisseria meningitidis* are the most common causes.
2. ***Viral*** Infections due to blood borne pathogens (hepatitis B virus, hepatitis C virus, and human immunodeficiency virus AIDS) remain the most common reported viral infections.
3. ***Fungal*** The dimorphic fungi (fungi which can exist as mold/hyphal/filamentous form or as yeast) are responsible for the greatest number of fungal infections.
4. ***Parasitic*** Malaria (*Plasmodium spp*), Giardiasis, isosporiasis (*Isospora belli*).

IV. RISK GROUP CLASSIFICATION

A. Risk Group 1 (RG1)

Microorganisms unlikely to cause human or animal diseases, and are not harmful to community. E.g. *E.coli* K-12, *Bacillus subtilis*, *Adeno associated viruses* (AAV type 1 to 4).

B. Risk Group 2 (RG2)

Pathogens/microorganisms moderately harmful to individuals or animals and less harmful to community or environment because there is limited risk of spread. These pathogens are not of serious hazard to laboratory workers or the community because effective treatment and preventive measures are available. E.g. *B.anthracis, C. tetani, C. botulinum, H. influenzae*, *H. pylori*, *Klebsiella*, *S.aureus*, Hepatitis A, B, C, D, and E virus.

C. Risk Group 3 (RG3)

Pathogens/microorganisms that are usually greatly hazardous to individual and animals but less harmful to community because these pathogens do not spread from person to person. Effective treatment and preventive measures are available. E.g. *Brucella, Burkholderia, Rickettsia, Yersinia pestis,* Bunya viruses, Rift Valley fever virus.

D. Risk Group 4 (RG4)

Pathogens highly hazardous to humans or animals as well as to the community because they can be transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually

available, e.g. herpes viruses (alpha) herpes virus simiae (Herpes B or Monkey B virus), Crimean-Congo hemorrhagic fever virus, arenaviruses.

V. LEVELS OF BIOCONTAINMENT LABORATORIES

Based on the degree of hazard associated with a microbial agent, there are four levels of biosafety containment laboratories, this categorization is dependent on laboratory practices and techniques, safety equipment, and facilities needed to protect against exposure.

A. BIOCONTAINMENT LABORATORIES 1 (BCL-1)

BCL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing. It deals with risk group 1 agents that are unlikely to cause diseases in healthy humans and are of minimal hazard to the laboratory and environment.

B. BIOCONTAINMENT LABORATORIES 2 (BCL-2)

BCL-2 also represents basic level of containment where lab practices, equipment, facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with risk group 2 agents that comprise those pathogens that can cause diseases in humans or animals but do not pose a serious hazard. Good microbiological practices with personal protective clothing are required. BSC Class 1 (Biosafety cabinet) is recommended.

C. BIOCONTAINMENT LABORATORIES 3 (BCL-3)

BCL-3 refers to containment level for special diagnostic, clinical and research services that deal with risk group 2-3 agents that have a potential for respiratory transmission, and which may cause serious and potentially lethal infection to humans and may spread to community. Special practices and special PPE are necessary with strongly recommended class 2 Biosafety cabinet i.e. BSC-2.

D. BIOCONTAINMENT LABORATORIES 4 (BCL-4)

BCL-4 is the maximum containment level applicable for work with risk group 4 agents that are dangerous and unusual agents that pose a high individual risk of life-threatening disease and high risk of spread for which no treatment

is available. The BCL-4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent the release of viable agents to the environment. BCL-4 should have Class 3 Biosafety cabinet or it can be a suit lab with BSC-2 facility.

VI. DIFFERENCE IN CONCEPT OF BIOSAFETY LEVELS AND RISK GROUPS

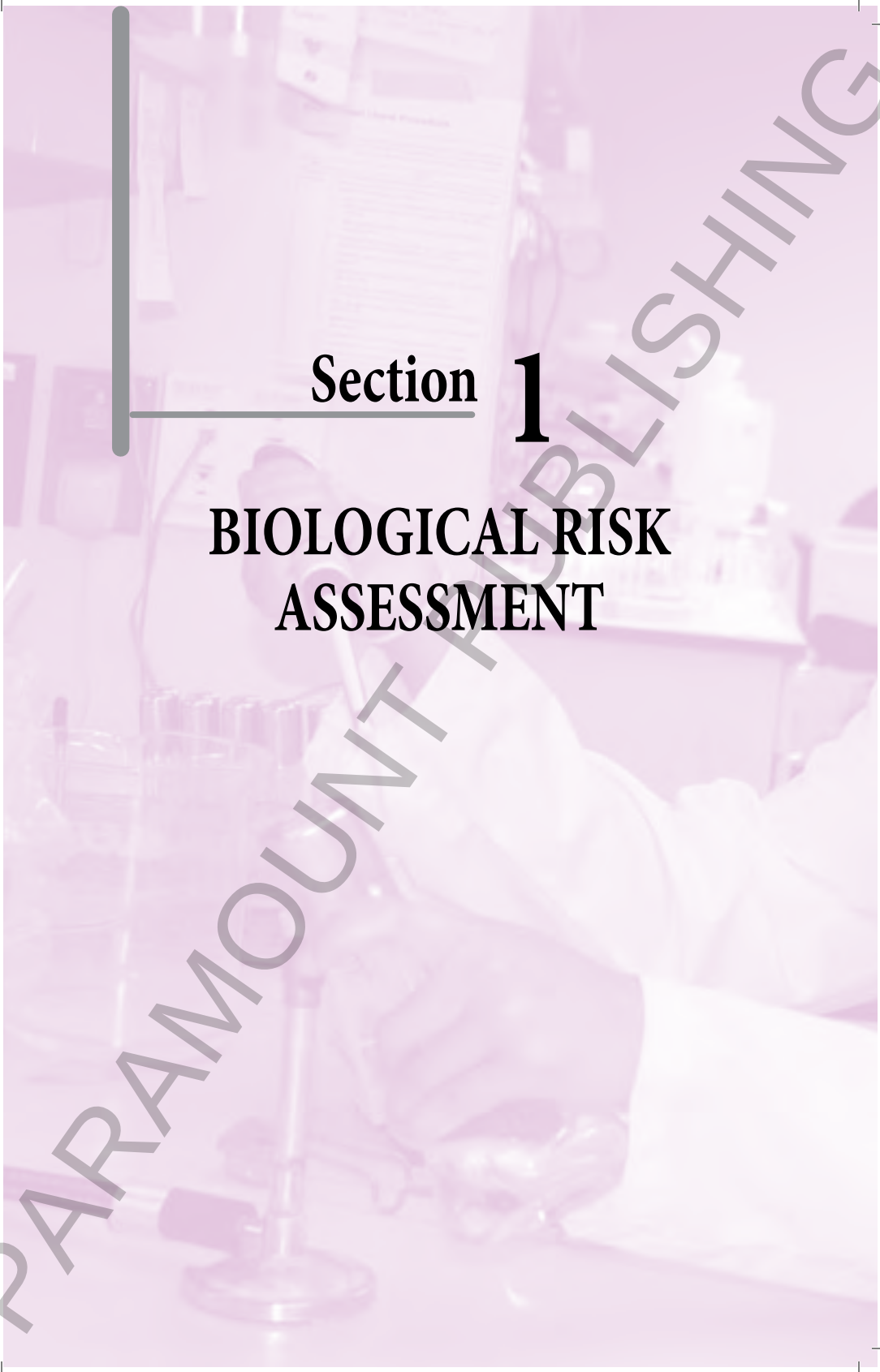
Risk groups and Biosafety levels are often misunderstood by the beginners, although both are different concepts. Biosafety levels (1, 2, 3, and 4) or containment levels refer to the type of laboratory on the basis of increasing level of hazard. Risk groups (1, 2, 3 and 4) refer to the categories of microbiological agents (i.e. bacteria, virus etc.) on the basis of increasing level of hazard associated with these agents to cause disease.

BSL-1 deals with **RISK GROUP 1** agents but not equals.

BSL-2 deals with **RISK GROUP 2** agents (or can even deal with risk group 3 agents with certain additional special practices or enhanced Biosafety practices).

BSL-3 deals with **RISK GROUP 3** agents (or can even deal with risk group 4 agents with certain additional special practices or enhanced Biosafety practices).

BSL-4 deals with **RISK GROUP 4** agents.



Section 1

BIOLOGICAL RISK ASSESSMENT

BIOLOGICAL RISK ASSESSMENT

I. RISK ASSESSMENT

Risk assessment is a process of judgment used to identify the hazardous characteristics of an infectious agent or material, laboratory procedures, practices and facility design that can result in a person's exposure to an agent, the likelihood that such exposure will cause a Laboratory-Acquired Infection (LAI), and the possible consequences of such an infection. The information identified by risk assessment will provide guidance for the selection of appropriate Biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs to individual, community and environment.

II. RISK ANALYSIS OF LABORATORY PROCEDURES AND WORK PRACTICES

According to risk assessment, the hazardous characteristics of laboratory procedures and practices is that mishandling or misuse may cause or transmit a Laboratory-Acquired Infection (LAI) to the worker. Risk assessment defines negligence among work practices as the most serious cause of laboratory acquired infections.

A. Modes of Laboratory-Acquired Infections

Laboratory Acquired Infections may occur by five different modes:

1. Inoculations with syringe, needles or other contaminated sharps.
2. Inhalation exposures to infectious aerosols.
3. Ingestion through mouth pippeting.
4. Animal bites and scratches.
5. Spills and splashes onto skin and mucous membranes.

Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of coworkers are basics for a laboratory staff in order to reduce the natural risks that attend work with hazardous agents.

III. RISK ANALYSIS OF SAFETY EQUIPMENT AND FACILITY SAFEGUARDS

Certain laboratory practices and procedures require specialized personal protective equipment in addition to safety glasses, laboratory gowns, and gloves.

Poor location, room air currents, decreased airflow, leaking filters, crowded work surfaces, and poor user technique reduces the controlling ability of a BSC.

Safety equipment such as Biological Safety Cabinets (BSC), centrifuge safety cups, and sealed rotors are used to provide a high degree of protection for the laboratory worker from exposure to microbial aerosols and droplets.

Sealed Rotor

Sealed Centrifuge Cups

Droplets are larger in size about 100 µm. They settle quickly and usually contaminate surfaces. Aerosols are smaller in size i.e. ≤100 µm, aerosols evaporate quickly and microorganisms are in dried form, thus, they travel further by air currents, and are most dangerous.

Safety equipment that is not working properly is unsafe. Proper maintenance and construction of laboratory facilities and designs is an important part of risk assessment. It is the responsibility of Institutional Biosafety Committee (IBS), Biosafety professionals and laboratory directors to assess risk associated with laboratory facilities and to provide satisfactory safety equipment and proper lab construction.

A. Five-Step Risk Assessment

1	• Identify agent hazards and perform an initial assessment of risk.
2	• Identify laboratory procedure hazards and assign appropriate risk groups.
3	• Make a determination of the appropriate biosafety level and select additinal precatioins indicated by the risk assessment.
4	• Evaluate the profictiencies of staff regarding safe practices and the reliability of safety equipment
5	• Review the risk assessment with a biosafety professional, subject matter expert, and the IBC

B. Responsibility of Risk Assessment in BSL-2 Labs of Pakistan

The laboratory director or Principal Investigator (PI), Biological Safety Professionals, Lab Manager and Pathologist if applicable by IBC are responsible for risk assessment, and to ensure that appropriate equipment and facilities are available to support the work being considered. Once performed, risk assessments should be reviewed routinely and revised when necessary.

A Biosafety committee comprising of at least 5 members shall be assigned in each health care institute. These members can be the employed staff workers, or external investigator.

For e.g. in a clinical laboratory, the MD, lab director, lab incharge, Biosafety officer and lab technologist can make up a team to work up for maintaining and implementing the organized Biosafety plan and policy for their institute. It will be helpful if they include two other related professionals from another institute or organization to give their opinion with respect to Biorisk issues.

Section 2

LABORATORY PRACTICES

Chapter

2

LABORATORY PRACTICES AND SAFETY BARRIERS

I. STANDARD MICROBIOLOGICAL PRACTICES

Standard microbiological practices or Good Microbiological Techniques (GMT) or Good Microbiological Laboratory Practices (GMLP) has two basic aims:

- To protect the lab workers and operators (students, teachers and technicians) from the very small possibility of infection.
- To save the product or practical investigations from becoming contaminated with microbes from external sources.

Standard microbiological practices are the most important component of Biosafety and are not restricted to be practiced only in higher level of containment laboratories. In fact, these practices must be followed from basic level of containment i.e. BSL-1 to maximum containment level BSL-4.

II. SPECIAL PRACTICES

For higher containment level, standard microbiological practices/GMT are not sufficient and are supplemented with additional special practices according to the likely hazard of work practice, procedures and risk group agent. These include immunization of staff, decontamination techniques, spill handling, and other procedures involving the manipulation of infectious materials.

III. PRIMARY BARRIERS

Primary barriers are the clothing or equipments that are designed in order to contain/block the exposure of hazard at its initial stage.

A. Types of Barriers

There are many types of primary barriers majorly categorized as follows:

1. PERSONAL PROTECTIVE EQUIPMENT

Personal Protective Equipment (**PPE**) refers to protective clothing that protects the wearer's body from physical or chemical injury and against biologically infectious material. The hazards and types of PPE appropriate for different body parts are listed in the following chart:

BODY PART	HAZARDS	PERSONAL PROTECTIVE EQUIPMENT
Eyes	Chemical or metal splash, dust, projectiles, gas and vapour, radiation.	Spectacles, goggles, face shields, visors
Head	Impact from falling or flying objects, risk of head bumping, hair entanglement.	Helmets and bump caps.
Respiratory tract	Dust, vapor, gas, oxygen-deficient atmospheres, aerosols.	Disposable filtering face piece or respirator, N 95, N99, PAPR.
Hands and arms	Abrasion, temperature extremes, cuts and punctures, impact, chemicals, electric shock, skin infection, disease or contamination.	Gloves, wrist cuffs.
Feet and legs	Electrostatic build-up, slipping, cuts and punctures, falling objects, metal and chemical splash, abrasion.	Safety boots and shoes with protective toe caps and penetration-resistant mid-sole.
Full body	Temperature extremes, adverse weather, chemical or metal splash, spray from pressure leaks or spray guns, impact or penetration, contaminated dust.	Lab coats, conventional or disposable overalls, boiler suits, specialist protective clothing.

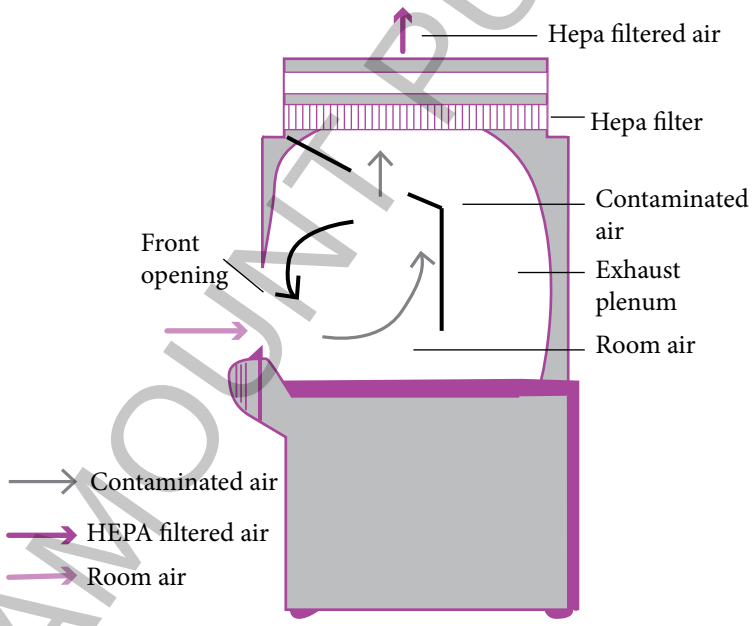
2. BIOLOGICAL SAFETY CABINETS

Biological Safety Cabinets (BSCs) or Biosafety Cabinets are designed to provide protection to the product, the user, and the environment when appropriate practices and procedures are followed. All aerosol generating or hazardous chemical material manipulation must be made inside these cabinets. There are three types of BSCs Class I, II, and III which are described below:

a) Class – I Biosafety Cabinets

Biosafety Cabinets are recommended to be used in BCL-2 labs. They provide protection to the personnel and the environment, but not to the product (pathogen or toxin).

They provide an inward flow of unfiltered air, similar to a chemical fume hood, which protects the worker from the material in the cabinet. The environment is protected by HEPA filtration of the exhaust air before it is discharged into the laboratory or ducted outside via the building exhaust.



The Class I Biosafety Cabinet

b) Class – II Biosafety Cabinets

Provide personnel, environment, and product protection.

- **Personnel Protection**: Air is drawn around the operator into the front grill of the cabinet, which provides personnel protection.

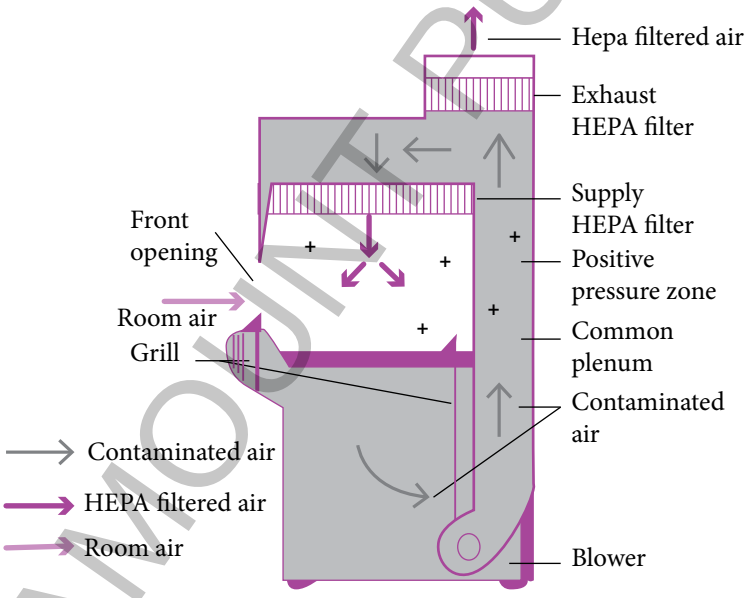
- **Product Protection:** The downward laminar flow of HEPA-filtered air within the cabinet provides product protection.
- **Environmental Protection**: The cabinet's air passes through the exhaust HEPA filter; it is contaminant-free and thus provides environmental protection.

(1) Classification

A1, A2, B1 and B2

(a) The Type A1 Cabinet (Type A)

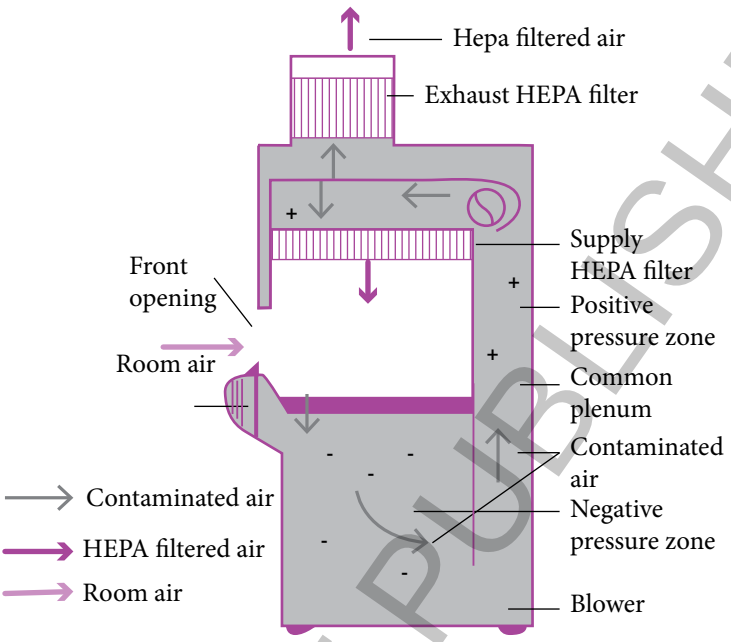
It has a minimum inflow velocity of 75 fl/min. Exhaust is drawn at the bottom of the cabinet where it rises to the top. At the top of the cabinet, 70% of the air recirculates through the supply HEPA filter, the other 30% of air exhausts through the exhaust HEPA filter. This cabinet is vented to the outside environment.



The Class II. Type A1 Biosafety Cabinet

(b) The Type A2 Cabinet (Type A/B3)

It has a minimum inflow velocity of 100 ft/min. A negative air pressure surrounds all contaminated capacity that is under positive pressure, identical to those of a Type A1 cabinet. Type A2 cabinet is also vented to the outside environment.



The Class II. Type A2 Biosafety Cabinet

(c) The Type B1 and B2 Cabinets

These have a minimum inflow velocity of 100 fl/min, Type B cabinets must be hard-ducted to an exhaust system rather than exhausted through a thimble connection. 60% of air from the rear grill is exhausted and only 40% is recirculated. Types B1 and B2 BSCs must be discharged directly to the outdoors via a hard connection. These hoods are commonly used in the hospital pharmacy, in addition to a wide range of clinical and research applications.

c) Class III Biosafety Cabinets (Class III glove boxes)

These are designed to work with risk group 4 infectious agents that require BSL4 containment laboratory. These cabinets provide maximum protection to the environment and the worker. The cabinet is gas-tight with a non-opening view window, and has rubber gloves attached to ports in the cabinet which allow for manipulation of materials in the cabinet. Air is filtered through one HEPA filter as it enters the cabinet, and through 2 HEPA filters before it exhausts to the outdoors. This type of cabinet provides the highest level of product, environmental, and personnel protection.

The common element to all classes of BSCs is the high efficiency particulate air (HEPA) filter. This filter removes particles of 0.3 microns with an efficiency of 99.97%. However, it does not remove vapors or gases.

d) Horizontal Laminar Flow Cabinets or Clean Air Benches

These are not BSCs, they only provide product protection because air is drawn through a HEPA-filter and is discharged across the work surface and toward the user, protecting only the product. These cabinets are usually made of stainless steel and exist in both ***horizontal*** and ***vertical*** configurations. They can be used for certain clean activities, such as dust-free assembly of sterile equipment or electronic devices. However, they should never be used when handling cell culture materials or potentially infectious materials, or as a substitute for BSC in research laboratories.

Laminar flow cabinets may have a UV-C germicidal lamp to sterilize the shell and contents when not in use.

e) Guidelines for Working in a Biological Safety Cabinet

(1) Inspect the air intake grilles for obstructions and foreign material and remove if necessary.

(2) Adjust view screen to proper height.

(3) Turn the cabinet on for at least 10 minutes prior to use, if the cabinet is not left running.

(4) Prepare a written checklist of materials necessary for the particular activity.

(5) Wash hands and arms with a mild soap. Put on a rear-fastening, long-sleeved gown with tight-fitting cuffs. Put on safety glasses and a pair (or two pairs) of high quality latex/nitrile gloves.

(6) Disinfect work surface with a suitable disinfectant.

(7) Place items into the cabinet so that they can be worked with efficiently without unnecessary disruption of the airflow, working with materials from the clean to the dirty side.

(8) Adjust the working height of the stool so that the worker's face is above the front opening.

(9) Delay manipulation of materials for approximately one minute after placing hands/arms inside the cabinet.

(10) Minimize the frequency of moving hands in and out of the cabinet.

(11) Do not disturb the airflow by covering any portion of the grillwork with materials.

(12) Work at a moderate pace to prevent the air flow disruption that occurs with rapid movements.

(13) Wipe the bottom and side of the hood surfaces with disinfectant when the work is completed.

f) Certification of the Biological Safety Cabinet

The BSC requires regular maintenance and certification by a professional technician to assure that it protects you, your experiments, and the environment. Each cabinet should be certified when it is installed, each time it is moved or repaired, and at least annually.

Certification is a series of performance tests on the BSC to confirm that it will provide the user and experimental material the protection for which it is designed. The airflow, filters, and cabinet integrity are checked to ensure that the cabinet meets minimum performance standards. Certification is arranged through each department and provided by an outside vendor.

BSCs intended for user protection and/or BSL2 work must be certified:

(1) After they are received and installed (before use with infectious materials).

(2) After filter changes.

(3) After being moved (even a few feet).

(4) Annually.

The formaldehyde gas production process must be used for BSC decontamination.

(1) Before any maintenance work requiring disassembly of the air plenum, including filter replacement.

(2) Prior to cabinet recertification.

(3) Before moving the cabinet to a new laboratory.

(4) Before discarding or salvaging.

g) Regional Conditions Regarding Biological Safety Cabinet

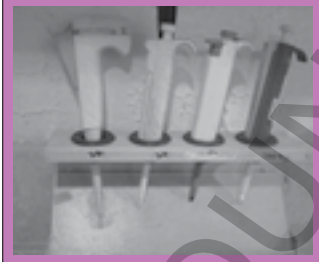
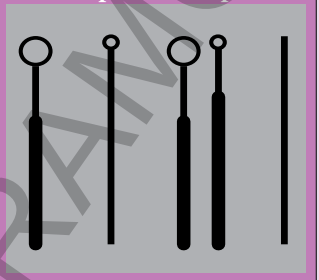
According to the latest surveys and prevailing conditions, it is found that the use of Biosafety Cabinet is not common in most labs of Pakistan. It would be 10% in all or less.

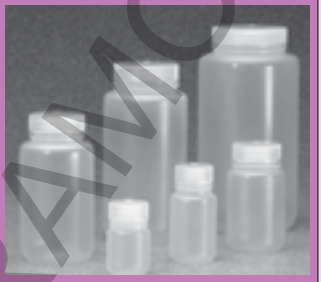
Admitting the financial obstacle and other local barriers, it is strongly recommended that institutional level certification or registration of labs must be made only if they are complying with standard set of work for Biosafety management.

3. OTHER SAFETY EQUIPMENTS

In order to minimize aerosol generation and to reduce the contact of worker with working agent, there are many safety equipments which should be used according to their manual directions and only by trained lab personnel.

A List of Safety Equipments with their Safety Feature is listed here:

SAFETY EQUIPMENT	HAZARD REDUCED	SAFETY FEATURES
Centrifuge cup 	Aerosol hazard.	To prevent aerosols from being released during centrifugation.
Pipetting aids 	Ingestion of pathogen or inhalation of aerosol by mouth, blowing or dripping of liquid, contamination of suction end of pipette.	Ease of use. Controls contamination of suction pathogens, protecting pippeting aid, user and vacuum line. Can be sterilized. Controls leakage from pipette tip.
Loop micro incinerators, disposable loops 	Spatter from transfer loops.	Shielded in open-ended glass or ceramic tube, heated by gas or electricity. Disposable, no heating necessary.

SAFETY EQUIPMENT	HAZARD REDUCED	SAFETY FEATURES
Sharps disposal containers Danger Box 	Puncture wounds	Autoclavable strong, puncture-proof/ Incineration
Transport containers 	Release of microorganisms	Tough Watertight primary and secondary containers to contain spills Absorbent material to contain spills
Autoclaves, manual or automatic	Infectious material (made safe for disposal or reuse)	Approved design Effective heat sterilization
Screw-capped bottles 	Aerosols and spillage	Effective containment

IV. SECONDARY BARRIERS

A. Facility Design and Construction (Secondary Barriers)

The design and construction of the Laboratory is called as secondary barrier because these layouts or facilities control the exposure of any infectious material or hazardous agent from escaping the lab and thus providing workers and environmental protection, as well as to protect persons or animals outside in the community from infectious agents that may be accidentally/ intentionally released from the laboratory.

The construction and facilities to be present in the laboratory are decided by Laboratory Director on the basis of risk analysis, and categorization of the laboratory into specific containment level according to the agents been manipulated. When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment which is usually recommended for higher level of containment i.e.; BSL3 or BSL4.

Section 3

BIOSAFETY GUIDELINES

Chapter

3

GUIDELINES FOR WORKING IN BIOSAFETY LEVEL 1 AND BIOSAFETY LEVEL 2

I. BIOSAFETY LEVEL 1

Biosafety Level 1 laboratory deals with those biological agents who are unlikely to cause diseases in healthy humans and are of minimal hazard to the laboratory environment and personnel. These agents are basically categorized under Risk Group 1.

BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using good microbiological practices. Special practices are not required, but may be used as determined by appropriate risk assessment as in case a risk group 2 organism comes under lab practice.

BSL-1 must meet the following standard practices, safety equipment, and facility requirements.

A. Standard Microbiological Practices

1. The laboratory supervisor must maintain going out and coming in to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory. Food and drinking water must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. **Mouth pippeting is prohibited**; mechanical pippeting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. The following sharp precautions must be practiced whenever needed:

   a) Careful management of needles and other sharps are of primary importance. Needles must not be bent, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

   b) Used disposable needles and syringes must be carefully placed in easily located puncture-resistant containers called Danger Boxes used for sharps disposal and not in ordinary waste container.

   c) Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

   d) Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan or forceps. Plastic ware should be substituted for glassware whenever possible.

6. Splashes and aerosols generating practices must be minimized.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. The specific disinfectant required depends upon the microorganisms studied, but for BSL-1 laboratories, a phenolic disinfectant is suitable.

8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport.

   a) Materials to be decontaminated outside of the laboratory must be placed in a durable, leak proof container and secured for transport.

   b) Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, provincial and National regulations.

9. Decontaminate waste daily.

10. Biohazard symbol must be posted at the laboratory door. When dealing with an infectious agent additional sign along with biohazard sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel.

11. Maintain insect and rodent control programme IPM (Integrated Pest Management)

12. The laboratory supervisor must ensure that laboratory workers receive appropriate training regarding Biosafety.

B. Safety Equipment (Primary Barriers)

1. PERSONAL PROTECTIVE EQUIPMENTS

The following PPEs are recommended for working in BSL1 lab:

a) Laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

b) Eyewear, when conducting procedures that may create splashes or aerosols.

c) Gloves must be worn to protect hands from exposure to hazardous materials.

2. SAFETY EQUIPMENTS

a) Special containment devices or equipment, such as BSCs are not generally required.

C. Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors.
2. Windows that can be opened and protected from fly screens.
3. An eyewash station should be located near the working area.
4. Sink for hand washing.
5. Floors, walls, and lab furniture must be washable.
6. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
7. Separate hanging areas for laboratory cloths.
8. Bench tops must be non absorbent and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

II. BIOSAFETY LEVEL 2

Biosafety Level 2 (BSL2) deals with those agents that are pathogenic and may cause moderate hazards to humans, animals, and the environment. But the hazard is not serious because effective treatment is available against these agents and there is a limited risk of spread.

A. Standard Microbiological Practices

Refer to BSL-1 Standard Microbiological Practices. In addition, following special practices are to be included when working at BSL-2.

B. Special Practices

1. A laboratory-specific Biosafety manual must be prepared and adopted as policy. The Biosafety manual must be available and accessible.
2. All persons entering the laboratory must be aware of the potential hazards associated with work practices and meet specific entry/exit requirements.
3. Laboratory personnel must be offered available immunizations for agents handled or potentially present in the laboratory.
4. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
   a) Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff, properly trained and equipped to work with infectious material.
   b) Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the Laboratory Biosafety Manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animal and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. *SAFETY EQUIPMENTS*

a) Properly maintained BSCs or other physical containment devices must be used whenever:

(1) Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pippeting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials.

(2) High concentrations or large volumes of infectious agents are used.

b) Leak proof vessels for collection and transport of infectious materials within facility.

c) Autoclave for safe sterilization.

d) Centrifuge cups to prevent aerosols from being released during centrifugation.

2. *PERSONAL PROTECTIVE EQUIPMENTS*

a) Protective laboratory coats, gowns, socks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas, e.g. cafeteria, library, and administrative offices. It is recommended that laboratory clothing not be taken home.

b) Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device.

c) Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. **Gloves must not be worn outside the laboratory.** In addition, BSL-2 laboratory workers should:

(1) Change gloves when contaminated or glove integrity is compromised, or when otherwise necessary.

(2) Remove gloves and wash hands when working with hazardous materials has been completed, and before leaving the laboratory.

(3) Do not wash or reuse disposable gloves. Dispose off used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

d) Eye, face and respiratory protection should be used as determined by the risk assessment.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors should be self-closing and have locks.
2. Laboratories must have a sink for hand washing. The sink may be manual, hands-free, or automatically operated. It should be located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
   a) Bench tops must be non absorbent to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
   b) Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior are not recommended. If present, they must be fitted with screens.
6. Vacuum lines should be protected with liquid disinfectant traps.
7. An eyewash station must be readily available.
8. There are no specific requirements for ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside the laboratory.
9. A method for decontaminating all laboratory wastes should be available in the facility (e.g. autoclave, chemical disinfection, incineration, or other validated decontamination method).
10. A Biosafety Cabinet must be installed.

Summary of Biosafety Levels 1, 2, 3 and 4

	RISK GROUP AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	SECONDARY BARRIERS/ FACILITIES
BSL-1	Not known to always cause diseases in healthy adults.	GMT: Controlled access. Proper hand washing. No eating, drinking, smoking etc. Sharps precautions. No mouth pippetting. Proper decontamination of work surface and waste. Biohazard symbols where necessary.	None required.	Laboratory bench, sink, eyewash, fly screen protected windows, floor walls, and furniture must be washable.
BSL-2	• Agents associated with human disease. • Routes of transmission include percutaneous injury, ingestion, • mucous membrane exposure.	BSL-1 practices plus: • Limited access. • Biohazard warning signs. • 'Sharps' precautions. • Biosafety manual.	Primary barriers: • Class II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols. • PPE: • Laboratory coats, gloves, face protection as needed.	BSL-1 plus: • Autoclave, refrigerator and centrifuge must be available. Inward directional airflow.

	RISK GROUP AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	SECONDARY BARRIERS/ FACILITIES
BSL-3	• Agent associated with human and animal diseases. • Disease may have serious or lethal consequences.	BSL-2 practices plus: • Controlled access. • Decontamination of all waste. • Decontamination of laboratory clothing before laundering.	Primary barriers: • Class I or II BSCs or other physical containment devices used for all open manipulation of agents. PPE: • Protective laboratory clothing; gloves; respiratory protection as needed.	BSL-2 plus: • Physical separation from access corridors. • Self-closing, double-door access • Exhaust air not recirculated. • Negative airflow into laboratory.
BSL-4	• Dangerous agents which pose high risk of life-threatening Disease. • Aerosol-transmitted laboratory infections have occurred; or related agents with unknown risk of transmission.	BSL-3 practices plus: • Clothing change before entering. • Shower on exit. • All material decontaminated on exit from facility.	Primary barriers: • All procedures conducted in Class III BSCs, or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit.	BSL-3 plus: • Separate building or isolated zone. • Dedicated supply and exhaust, vacuum, and decontamination systems. • Other requirements according to work practice.

Section 4

ROLES AND RESPONSIBILITIES OF LABORATORY PERSONNEL

Chapter

4

RESPONSIBILITIES

I. ROLES

A. Principal Investigator/Biosafety Officer

The responsibilities of Biosafety officer BSO are as follows:

1. Be well-trained in organizing Biosafety programme according to regional and national policies and guidelines.
2. Develop protocols and procedures to address issues of Biosafety.
3. Develop, implement, and maintain the institutions Biosafety programme.
4. Provide adequate training to workers handling the biohazardous materials.
5. Advise laboratory workers and waste handlers/janitorial staff about proper waste disposal methods.
6. Assist in the development of emergency plans for handling accidental spills and personnel contamination.
7. Conduct inspection of lab practices and facilities weekly or daily as required.
8. Work in collaboration with Principal Investigator and Institutional Biosafety Committee.
9. Assist in dealing with infectious agents; human blood, body fluids, tissue, or cell culture; or select agents and toxins.

B. Institutional Biosafety Committee

The Biosafety committee should be first organized at each institution comprising of trained professionals in Biosafety. This committee can comprise of Biosafety officer (BSO), lab supervisor, principal investigator, dean and/or director of the institution. The Institutional Biosafety Committee (IBC) has

following responsibilities to fulfill regarding Biosafety maintenance:

1. Provide necessary training in Biosafety maintenance to lab staff.
2. Determine the containment level of lab according to risk assessment of work practices.
3. Implement policies to minimize the exposure of hazardous lab materials.
4. Authorize expert personnel to access the facilities, procedures, practices.
5. Maintain health surveillance of laboratory workers.
6. In case of change of work practices to higher level of containment, proper risk analysis and enhancement of lab facilities to higher level of containment should be made.
7. Follow and implement the NIH guidelines to procedures, practices, facilities and other issues regarding Biosafety.

C. Pakistan Biological Safety Association

1. Pakistan biological safety association (PBSA) is formulated for providing a comprehensive knowledge related to Biosafety and Biosecurity issues in Pakistan.
2. Establish a group of trained professionals in the field of Biosafety, who will develop ways for risk assessment, guidelines for the use of safety equipments, and containment facilities in order to minimize LAIs and simultaneously any infectious exposure to the community in the country.
3. To maintain Biosafety standard in accordance with national and international Biosafety protocols (CWA15793) LBM 3rd Edition.
4. Provide awareness to public about importance of Biosafety via newsletters, seminars, workshops, Biosafety manuals and International conferences.

D. Deans, Directors and Microbiologists

1. Appoint Biosafety officer (trained according to national and regional guidelines of Biosafety policy) for maintenance of Biosafety issues in the institution.
2. Must organize an Institutional Biosafety Committee (IBC) comprising of at least five members professionally experienced in Biosafety.
3. Cooperate with PBSA in Biosafety related issues and updates.
4. Ensure that lab staff and members are receiving proper training to meet Biosafety criteria according to standard level (CWA 15793).

5. Provide sufficient facilities to maintain specific level of containment laboratory as required for work practices.

E. Laboratory Employees

1. Receive appropriate training and instruction in Biosafety and Biosecurity issues before access to laboratory as given by IBC or Biosafety officer.
2. Understand the need and outcomes of mishandled biological agents, and the potential risks associated with their exposure.
3. Revise and practice the emergency response procedures in case of spill or accidental release of biohazardous material.
4. Follow all laboratory practices and protocols, and comply with all applicable guidelines and policies.
5. Complete any necessary medical surveillance.
6. Report all thefts, security incidents, accidents, spills, or contamination incidents to supervisor.

F. Health Care Provider

1. In order to control biohazards, Health care provider should maintain effective immunoprophylactic and occupational health status.
2. Workers should be educated about the biohazards to which they may be occupationally exposed, the types of exposures that place their health at risk, the nature and significance of such risks,
3. Appropriate first aid and follow-up for potential exposures should be regulated.
4. That information should be reinforced annually, at the time of any significant change in job responsibility, and following recognized and suspected exposures.
5. The health care provider should review the worker's previous and present medical status, current medications, allergies to medicines, animals, and other environmental proteins, and prior immunizations.
6. Commercial vaccines should be made available to workers to provide protection against infectious agents to which they may be occupationally exposed.
7. Routine, periodic medical evaluations generally are not recommended; however, limited periodic medical evaluations or medical clearances targeted to job requirements may occasionally be made for an occupationally acquired infection.

II. EXPOSURE CONTROL MEASURES

A. Biological Spills

Biohazardous material's spill can cause serious health hazard within lab premises to the workers as well as to the other workers around lab surroundings. A spill kit should be kept in each laboratory in order to provide immediate aid in case of emergency.

1. SPILL KIT CONTENTS

The lab incharge should prepare a spill kit containing:

a) An absorbent material,

b) Concentrated disinfectant,

c) Rubber gloves,

d) Autoclave bags,

e) Sharps container, and

f) Forceps to pick up sharps/broken glass.

2. BIOSAFETY LEVEL 1 SPILL CLEANUP GUIDELINES

a) Mark and restrict the spilled area.

b) Wear lab coat and gloves.

c) Use forceps to pick up broken glass and discard into sharps container.

d) Cover spilled material with paper towels.

e) Add diluted disinfectant in sufficient quantity to ensure effective microbial inactivation.

f) Dispose of towels in biohazard waste container and autoclave if necessary.

g) Wipe spill area with diluted disinfectant.

h) Wash hands with soap and water when finished.

i) Inform lab supervisor about the spill.

j) Record the incidence of spill with the name of organism in use.

3. BIOSAFETY LEVEL 2 SPILL CLEANUP GUIDELINES

a) Mark and restrict the spilled area.

b) Wear gloves and protective clothing (as necessary) like face shield or eye protection.

c) Keep other workers out of the area to prevent spreading of spilled material.

d) Specify and restrict the area by posting warning signs.

e) Remove any contaminated clothing, ensuring that clothing is not pulled over the face, and put into a biohazard bag for later autoclaving.

f) Inform and seek assistance of lab supervisor or Biosafety officer.

g) Pick up broken glass with forceps and dispose into sharps container.

h) Cover the spill with paper towels or absorbent material.

i) Add appropriately diluted disinfectant.

j) After at least 20 minutes contact time, pick up the paper towels and re-wipe the spill area with diluted disinfectant.

k) Collect all contaminated materials into biohazard waste container and autoclave.

l) Wash hands with soap and water.

4. SPILL INSIDE A BIOSAFETY CABINET

a) Alert the other laboratory employees.

b) Leave the cabinet turned on.

c) Wear gloves and labcoat and other personal protective clothing as required.

d) Cover the spilled area with paper towels.

e) Spray or wipe cabinet walls, work surfaces, and equipment with disinfectant such as 70% ethanol. Allow at least 20 minutes contact time.

f) Ensure that no paper towels or solid debris are blown into area below the grille.

g) Discard all clean-up materials into biohazard waste container.

h) Wash hands and exposed skin areas with soap and water.

i) The BSO Biosafety officer/ Lab supervisor should be notified of the spill immediately after the event.

III. EXPOSURE TO BIOLOGICAL MATERIALS

In case of an accident with biohazardous material or exposure to a biohazardous agent, the following emergency response procedures should be followed:

A. Skin Exposure

1. Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur. Shirts should be cut off.
2. Vigorously wash contaminated skin for 1 minute with soap and water.

3. Seek medical aid if necessary.
4. Inform the laboratory's supervisor or principal investigator.

B. Eye Exposure

1. Immediately flush the eye with a gentle stream of clean, temperate water for 15 minutes. Hold the eyelid open. Be careful not to wash the contaminant into the other eye. Use emergency eyewash if one is accessible.
2. Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur. Shirts should be cut off.
3. Seek medical aid if necessary.
4. Inform the laboratory's supervisor.

C. Ingestion or Inhalation

1. Move to fresh air immediately.
2. Seek medical attention available at the campus.
3. Do not induce vomiting unless advised to do so by a health care provider.
4. Inform the laboratory's supervisor or a Biosafety officer.

D. For Severe Injuries

1. Immediately seek medical assistance and if not available within the institution then transfer the victim to the nearest emergency center.
2. Accompany the injured person and provide information to the doctor about the incident
3. Report accident to the lab supervisor as well as to the Biosafety officer.

IV. FIRES INVOLVING BIOLOGICAL MATERIALS

- ✦ Without placing yourself in danger, put biological materials in secure location, such as incubator or freezer.
- ✦ In case of small fire like paper from burner or any other small object, pour sufficient amount of water over it to put off the fire or use fire extinguisher if available.
- ✦ Major cases should not be handled by one self.
- ✦ Activate the building fire alarm if present.
- ✦ Leave the building at once.
- ✦ Call the fire department from a safe location.

Section 5

DISINFECTION AND DECONTAMINATION

Chapter

5

DISINFECTION AND DECONTAMINATION

I. IMPORTANT TERMINOLOGIES

Disinfectants (Bactericide, Germicide)

These are substances that are applied to non-living objects to destroy vegetative microorganisms that are living on the objects. It is less effective than sterilization.

Sterilizers (sterilization)

These are substances that kill all forms of microbial life, such as fungi, bacteria, viruses, spore forms, etc. present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media. Sterilization comprises the application of heat, chemicals, irradiation, high pressure, and filtration.

Sanitizers

These are similar in function to disinfectant. Sanitizers reduce the number of vegetative bacteria only.

Antibiotics

These substances destroy microorganisms within the body.

Antiseptics

These destroy microorganisms on a living tissue.

II. TYPES OF DISINFECTANTS

DISINFECTANT	AVAILABILITY	ADVANTAGES	DISADVANTAGES
Chlorine	Liquid, powder, or tablet forms are available.	Effective against vegetative bacteria, mycobacterium, viruses, and fungal spores.	Corrosive, neutralized by organic material.
Iodine	Aqueous solutions as tinctures (solution in alcohol) or as iodophores (providone iodine complex).	Effective against enveloped viruses, vegetative bacteria, fungi, and to some extent, against mycobacteria, non-enveloped viruses, and bacterial spores.	Causes staining of treated material, corrosive, neutralized by organic material.
Alcohol	Ethyl or isopropyl alcohol. Seventy percent solution in water.	Enveloped viruses, vegetative bacteria, fungi, mycobacteria, variable against non enveloped viruses, and no activity against bacterial spores.	Quickly evaporates thus longer contact time can't be achieved.
Phenolics	Wide varieties are available in combination with detergents.	Enveloped viruses, vegetative bacteria, variable against fungi, mycobacteria, no activity against bacterial spores.	Toxic, neutralized by hard water, produces pungent smell.
Quaternary Ammonium Compounds	Wide varieties are available in combination with detergents.	Effective against gram positive bacteria.	Limited activity against gram negative and enveloped viruses.

DISINFECTANT	AVAILABILITY	ADVANTAGES	DISADVANTAGES
Glutaraldehyde	Two percent solution with activator.	Broad spectrum of activity against all types of microorganisms including non enveloped viruses, mycobacteria and bacterial spores. Rapid activity. Non corrosive. Not neutralized by organic material.	Activated product has limited shelf life. May produce adverse health affects like mucous membrane irritation, contact dermatitis, occupational asthma.
Formaldehyde	Solid paraformaldehyde (flakes or tablets) or as liquid formalin.	Broad spectrum of activity against all types of microorganisms including non enveloped viruses, mycobacteria and bacterial spores. Rapid activity. Non corrosive. Not neutralized by organic material.	Adverse health effects.
Hydrogen Peroxide	Available in vaporous form.	Rapid decontamination. Compatible with electronics, no residue or toxic byproducts.	
Chlorhexidine	Available in gaseous and vaporous form.	Rapid decontamination, rapid aeration, Compatible with electronics, no residue or toxic byproducts.	

Section 6

LABORATORY HAZARDS

Chapter

6

CHEMICAL, FIRE AND ELECTRICAL SEFETY

In addition to hazardous microorganisms there are other hazardous agents in laboratory like chemicals, fire, electrical plugs, heavy objects, noise etc. These agents can also cause serious health problems among laboratory workers. It is important for a lab worker to have complete information about all the hazards present in his work environment and should be well-trained to handle any material or object in a proper manner.

I. CHEMICAL HAZARDS

Chemicals that are used and stored in laboratory have the potential to cause harm to its handler. Some chemicals adversely affect the health of those who handle them or inhale their vapors. The toxicity of certain chemicals can affect the respiratory system, blood, lungs, liver, kidneys and the gastrointestinal system as well as other organs and tissues. Some chemicals are known to be carcinogenic or teratogenic.

A. General Rules Regarding Chemical Management

1. Chemicals that are necessary for daily use should be stored in the laboratory. Stock amounts of chemicals should be kept in specially designated rooms or storage areas.
2. Chemicals should not be stored in alphabetical order because in doing so certain reacting chemicals come aside of each other which may cause serious fire reaction or explosion upon interaction.
3. Azides often used in antibacterial solutions should not be allowed to come into contact with copper or lead (e.g. in waste pipes and plumbing), as they may explode violently when subjected even to a mild impact.
4. Old or dried Ethers (crystals) are extremely unstable, and potentially explosive, thus, they must be periodically discarded safely and changed with fresh ones.

SUBSTANCE CATEGORY	MISMATCHED SUBSTANCES
Alkali metals, e.g. sodium, potassium, cesium and lithium.	Carbon dioxide, chlorinated hydrocarbons, water.
Halogens	Ammonia, acetylene, hydrocarbons.
Acetic acid, hydrogen sulphide, aniline hydrocarbons, sulphuric acid.	Oxidizing agents, e.g. chromic acid, nitric acid, peroxides, permanganates.

5. Perchloric acid, if allowed to dry on woodwork, brickwork or fabric, will explode and cause a fire on impact.
6. Picric acid and picrates are associated with heat generating hazard.
7. Appropriate spillage charts should be displayed in a prominent position in the laboratory.
8. Spill kit should be always available and should be within reach.
9. Additional requirements to deal with chemical spill include paper towels, Soda ash (sodium carbonate, Na_2CO_3) or sodium bicarbonate ($NaHCO_3$) for neutralizing acids and corrosive chemicals, sand (to cover alkali spills) and non-flammable detergent.

II. FIRE HAZARDS

Apart from chemical hazards, fire possesses another major hazardous feature for laboratory worker.

In order to minimize the risk associated with fire, following measures should be adopted while working in the laboratory:

- Immediate action in case of fire and the use of fire-fighting equipment is desirable.
- Fire warnings, instructions and escape routes should be displayed prominently in each room and in corridors and hallways.
- Electrical circuit must not be overloaded.
- Proper electrical maintenance, e.g. proper insulation on cables.
- Excessively long gas tubing or long electrical leads must be avoided.
- Equipment should never be left switched on unnecessarily.
- Working on open flames should be handled with perfect care.

- Deteriorated gas tubing should be replaced.
- Proper handling and storage of flammable or explosive materials.
- Sparking equipment must never be placed near flammable substances and vapors.
- Proper or adequate ventilation should be available.
- Fire-fighting equipment like hoses buckets (of water or sand) and a fire extinguisher should be placed near room doors and at strategic points in corridors and hallways.

III. ELECTRICAL HAZARDS

- All electrical switches, plug, and equipment must be inspected and tested regularly, including earthing/grounding systems.
- Circuit-breakers should be installed in laboratory electrical circuits to protect wiring from being overloaded with electrical current and hence to prevent fires.
- Electrical equipment should not be placed near air conditioners, pipelines as these pipes may leak and damage electrical equipment and may even cause fire.

All laboratory electrical equipment should be earthed/grounded, preferably through three-prong plugs.

Section 7

LABORATORY BIOSECURITY CONCEPTS

Chapter

7

PRINCIPLES OF LABORATORY BIOSECURITY

I. THE CONCEPT OF BIOSECURITY

Biosafety and Biosecurity are different concepts with respect to their goals.

According to WHO Biosafety guidelines:

'Laboratory Biosafety' is the term used to describe the containment principles, technologies and practices that are implemented to prevent ***unplanned*** exposure to pathogens and toxins, or their accidental release.	***'Laboratory Biosecurity'*** refers to institutional and personal security measures designed to prevent the loss, theft, misuse, diversion or ***planned*** release of pathogens and toxins.

II. BIOSECURITY

Biosecurity is a set of preventive measures designed to reduce the risk of transmission of infectious diseases, infected pests and living modified organisms. These preventative measures comprise systems and laboratory practices to prevent the use of dangerous pathogens and toxins for use as well as by customs agents and agricultural and natural resource managers to prevent the spread of these biological agents in natural environment.

When talking about security it is usually thought of in terms of 'Guards, Gates, and Guns'. In the same way, Biosecurity encompasses the security that requires the cooperation of scientists, technicians, policy makers, security engineers, and law enforcement officials.

A. Components of a Laboratory Biosecurity Programme

These include:

1. Physical Security
2. Personnel Security
3. Material Control and Accountability

4. Transport Security
5. Information Security
6. Programme Management

III. BIOSECURITY AND BIOTERRORISM

The concept of Biosecurity arouse from Biosafety and is intended to provide 'product protection' from intentional release or theft of a biologically hazardous agent from laboratory in order to bring harm to humanity, animal life, community or the environment.

The incidences of bioterrorism in the past years have created a need to develop effective Biosecurity programme and implement it on immediate basis according to risk assessment of the type of agent handled in lab and level of containment.

IV. DEVELOPING A BIOSECURITY PROGRAMME

In order to ensure effective Biosecurity programme in laboratory, the Biosafety Officer, Institutional Head, Principal Investigator, and the Biosafety Authority must maintain the Biosecurity plan by keeping following points ahead;

- Maintain restricted personnel access to pathogens and toxins.
- Train personnel with access regarding the description of use, storage, handling of hazardous material.
- Keep information of professional and ethical record of personnel with access.
- Keep an updated inventory of chemicals, stocks, storage equipments, cultures, growth media, pathogenic samples, and toxins.
- Keep laboratory doors closed and locked when unoccupied.
- Keep stocks of pathogenic organisms, toxins, and hazardous chemicals locked when the laboratory is unoccupied.
- Notify Institutional Head, or Biosafety Officer if materials are damaged or missing from laboratories.
- Inspect all packages arriving into the laboratory.
- Laboratory Biosecurity training should be given to all lab staff and make sure that they understand the need for protection of such materials and consequences of their release.
- Decontaminate materials and work surfaces daily and after completing work.
- Ensure proper and safe storage of biologically hazardous materials.
- Discuss other security-specific requirements with your supervisor and colleagues.
- Do not allow strangers to enter in the lab premises.

Section 8

APPENDICES

Chapter

8

APPENDICES

Appendix A - Biosafety Inspection Checklist

STANDARD MICROBIOLOGICAL PRACTICES			
S. NO	QUESTIONS	YES	NO
1	Do workers wash their hands after working with biohazardous materials, after removing gloves, and before leaving the lab?		
2	Are all procedures performed carefully in a manner to minimize the creation of splashes or aerosols?		
3	Are work surfaces decontaminated with an effective disinfectant, after completion of work or at the end of the day, and especially after overt spills or splashes of biohazardous materials?		
4	Is mouth pipetting prohibited and are mechanical pipetting devices used?		
5	Is eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption prohibited in the lab?		
6	Are all syringes/needles/sharps disposed of in rigid, puncture-resistant, leak-proof containers?		
7	Are all wastes that are contaminated with biohazardous materials autoclaved or decontaminated with an effective disinfectant before discarding?		
8	Is there an SOP for proper autoclave use posted next to the autoclave or for other equipments?		

STANDARD MICROBIOLOGICAL PRACTICES			
S. NO	QUESTIONS	YES	NO
	SPECIAL PRACTICES		
9	Are all workers informed and well-trained for occupational health hazards and immunoprophylaxis?		
10	Are lab personnel required to read and fill out Lab-Biosafety Checklist ?		
11	Are needle-locking syringes or safety hypodermic needles used when appropriate?		
12	Do lab personnel understand that used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated?		
13	Do lab personnel use mechanical means, such as a brush and dustpan, tongs, or forceps to clean up broken glassware?		
14	Are cultures, tissues and other biohazardous materials placed in a container with a cover that prevents leakage during collection, handling, processing, storage, or transport?		
15	Do lab personnel notify the supervisor immediately if there are spills and accidents that result in exposures to biohazardous materials?		
16	Is medical follow-up obtained if appropriate?		
17	Are all laboratory personnel and particularly women of childbearing age provided with information regarding immune competence and conditions that may affect them?		
	SAFETY EQUPIMENT		
18	If there is a Biological Safety Cabinet in the lab, has it been certified within the past year?		
19	Is the Biological Safety Cabinet free of equipment or supplies that can block the air grills and disrupt proper airflow?		
20	Is a Biological Safety Cabinet used for all procedures with a potential for creating biohazardous aerosols or splashes?		

STANDARD MICROBIOLOGICAL PRACTICES			
S. NO	QUESTIONS	YES	NO
21	Are other safety and precautionary measures adapted while grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of biohazardous materials in case a Biosafety cabinet is not present?		
22	Are autoclaves and other pressure vessels regularly inspected?		
23	Are plastics used instead of glass where feasible?		
24	Are portable fire extinguishers maintained fully charged and in working order, and kept in designated places at all times?		
25	Is equipment for use or storage of biohazardous materials (i.e. refrigerator, freezers) labeled with a biohazard symbol?		
26	When lab personnel centrifuge biohazardous materials, do they use sealed rotor heads or centrifuge safety cups and open the rotors or safety cups only in a Biological Safety Cabinet?		
PERSONAL PROTECTIVE EQUIPMENT (PPE)			
27	Do personnel wear lab coats whenever they are in the lab and remove them before leaving the lab?		
28	Are personnel prohibited from taking their lab coats home for laundering?		
29	Do personnel wear gloves to prevent contact with biohazardous materials?		
30	Do personnel remove gloves before touching 'clean' surfaces (keyboards, telephones, elevators, etc.) and before leaving the lab?		
31	Are safety glasses, goggles and shields (visors) provided?		
32	When biohazardous materials must be manipulated outside a Biological Safety Cabinet, do personnel use eye and face protection?		
33	Are alternatives to powdered latex gloves available for personnel with latex sensitivity?		

STANDARD MICROBIOLOGICAL PRACTICES			
S. NO	QUESTIONS	YES	NO
	LAB FACILITIES		
34	Is a BIOHAZARD sign posted on the lab entrance door which includes the Biosafety level, any required immunizations, emergency contact numbers, and any personal protective equipment that must be worn in the lab?		
35	Are there any structural defects in floors?		
36	Is the working space adequate for safe operation?		
37	Is the general illumination adequate (e.g. 300–400 lx) and all areas well-lit, with no dark or ill-lit corners in rooms and corridors?		
38	Is the storage facility for bulk flammable liquids separated from the main building?		
39	Do the premises meet national and local building requirements		
40	Are the premises constructed and maintained to prevent entry and harborage of rodents and arthropods?		
41	Does the lab contain a sink for hand washing?		
42	Are bench tops impervious to water and resistant to moderate heat and the chemicals used to decontaminate the work surfaces and equipment?		
43	Is there a fire alarm system?		
44	Is an independent power support unit provided in case of power breakdown?		
45	Are carpets and rugs kept out of the lab?		
46	Are chairs and other furniture used in the lab covered with a non-fabric material that can be easily decontaminated?		
47	Is there eyewash available to laboratory personnel?		
48	Are storage facilities, shelves, etc. arranged so that stores are secure against sliding, collapse or falls?		

STANDARD MICROBIOLOGICAL PRACTICES			
S. NO	QUESTIONS	YES	NO
	SANITATION AND STAFF FACILITIES		
49	Are clean and adequate toilet (WC) and washing facilities provided separately for male and female staff?		
50	Is there an occupational health service?		
51	Is there an immunization programme relevant to the work of the laboratory?		
52	Is drinking-water available?		
53	Are first-aid boxes provided at strategic locations?		
54	Is there accommodation (e.g. lockers) for street clothing for individual members of the staff?		
55	Are noise levels acceptable?		
	HEATING AND VENTILATIONS		
56	Is there a comfortable working temperature?		
57	Are blinds fitted to windows that are exposed to full sunlight?		
58	Does mechanical ventilation compromise airflows in and around Biological Safety Cabinets and fume cupboards?		
59	Is the ventilation adequate, e.g. at least six changes of air per hour, especially in rooms that have mechanical ventilation?		
60	Are there HEPA filters in the ventilation system?		
	BLOOD-BORNE PATHOGENS		
61	Have lab personnel been offered and received appropriate immunizations for the agents potentially present in the lab (e.g. hepatitis B) Or declined in writing?		
62	Do the lab personnel have access to the Blood-borne Pathogens Exposure Control Plan?		
63	Have all lab personnel with the potential for exposure to Blood-borne Pathogens or other potentially infectious materials completed Blood-borne Pathogens Training programme?		

Appendix B - Acronyms

ABSA American Biological Safety Association

ABSL Animal Biosafety Level

ACIP Advisory Committee on Immunization Practices

BMBL Biosafety in Microbiological and Biomedical Laboratories

BSC Biological Safety Cabinet

BSL Biosafety Level

BSL-3-Ag BSL-3-Agriculture

BSO Biological Safety Officer

CAV Constant Air Volume

CDC Centers for Disease Control and Prevention

CNS Central Nervous System

CSF Cerebrospinal Fluid

DHHS Department of Health and Human Services

DoC Department of Commerce

DOD Department of Defense

DOL Department of Laboratory

EBV Epstein-Barr Virus

EPA Environmental Protection Agency

EtOH Ethanol

FDA Food and Drug Administration

FFI Fatal Familial Insomnia

FMD Foot and Mouth Disease

FMDV Foot and Mouth Disease Virus

GI Gastrointestinal Tract

HEPA High Efficiency Particulate Air

HIV Human Immunodeficiency Virus

HVAC Heating, Ventilation, and Air Conditioning

IATA International Air Transport Association

IBC Institutional Biosafety Committee

ID Infectious Dose

ID50 Number of organisms necessary to infect 50% of a group of animals

IgG Immunoglobulin
IND Investigational New Drug
IPM Integrated Pest Management
LAI Laboratory-Associated Infections
LD Lethal Dose
LMW Low Molecular Weight
MMWR Morbidity and Mortality Weekly Report
MPPS Most Penetrating Particle Size
NaOCl Sodium Hypochlorite
NaOH Sodium Hydroxide
NBL National Biocontainment Laboratory
NIH National Institutes of Health
NIOSH National Institute for Occupational Safety and Health
OBA NIH Office of Biotechnology Activities
OIE World Organization for Animal Health
OPV Oral Poliovirus Vaccine
OSHA Occupational Safety and Health Administration
PAPR Positive Air-Purifying Respirator
PPM Parts Per Million
Prp Prion Protein
RBL Regional Biocontainment Laboratory
SARS Severe Acute Respiratory Syndrome
SARS-CoV SARS-Associated Coronavirus
SCID Severe Combined Immune Deficient
SOP Standard Operating Procedure
TLV Threshold Limit Values
UV Ultraviolet
UPS Uninterrupted Power Supply
WHO World Health Organization

Appendix C - List of contact details of Biosafety organizations, official personnel in major cities of Pakistan.

P.B.S.A

Pakistan Biological Safety Association

FL3/3

Block 4

Gulshan-e-Iqbal

Karachi

Email: pbsa.pak@gmail.com

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Patron in Chief, PBSA

Scientist, Ph.D. from University of Edinburgh

Former Advisor Science in COMSTECH

Dr. Shamsul Arfin Qasmi

MBBS, RBP (USA), M.Phil (UOK), Dip Hem (BMU)

Associate Professor

Sir Syed Medical & Dental College

Former General Secretary PBSA

Member Executive Council PBSA

Dr Aamir Ikram

MCPS, FCPS, RBP (USA)

Consultant Microbiologist, Associate Professor of Pathology

Founder PBSA

Mrs. Areej Mehtab

Research Associate at MED PATH Laboratory and Diagnostic Center

Consultant Microbiologist & Lecturer

M.Sc. in Microbiology

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